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PERKINSUS (PROTOZOA: APICOMPLEXA) INFECTIONS IN ABALONE FROM SOUTH AUSTRALIAN WATERS

by P. J. O'DONOGHUE*, P. H. PHILLIPS* & S. A. SHEPHERD†

Summary

O'DONOGHUE, P. J., PHILLIPS, P. H. & SHEPHERD, S. A. (1991) *Perkinsus* (Protozoa: Apicomplexa) infections in abalone from South Australian waters. *Trans. R. Soc. S. Aust.* **115**(2), 77–82, 31 May, 1991.

A total of 234 abalone were collected from nine sites in South Australian coastal waters and examined for infections with the protozoan parasite *Perkinsus* sp. Infections were detected in ten *Haliotis laevigata* from one location in Gulf St Vincent and in nine *H. rubra* from another location in Spencer Gulf. All infections were characterized by the presence of macroscopic necrotic nodules (0.5–8.0 mm in diameter) in the adductor muscles and mantle. Microscopic examination revealed the nodules to contain variable numbers of host amoebocytes and numerous developmental stages of the parasite, including single ovoid trophozoites (10.0–17.5 μm) and larger rounded schizonts (12.5–35.0 μm) containing vacuolated merozoites. The morphological and ultrastructural characteristics of the parasites were similar to those previously described for *P. olseni*. A total of 240 Pacific oysters *(Crassostrea gigas)* were also examined from four commercial farms in neighbouring coastal waters but no *Perkinsus* infections were detected.

KEY WORDS: Apicomplexa, *Perkinsus*, abalone, *Haliotis*, morphology.

Introduction

Two abalone species are fished commercially from South Australian coastal waters; blacklip abalone, *Haliotis rubra* Leach, and greenlip abalone, *H. laevigata* Donovan. Since 1972, licensed divers have reported the occurrence of yellowish pustules in the flesh of *H. rubra* collected near Neptune Island in Spencer Gulf. The pustules render the flesh of the abalone unacceptable for processing and marketing. Microscopic examination revealed the pustules to be caused by a protozoan parasite, *Perkinsus olseni* Lester & Davis, 1981. Only two other *Perkinsus* spp. have been described; *P. marinus* from the American oyster *Crassostrea virginica* (Mackin *et al.* 1950; Perkins 1969) and *P. atlanticus* from the clam *Ruditapes decussatus* (Azevedo 1989). In recent years, local divers have become increasingly concerned with dwindling stocks of *H. laevigata* along the western shore of Gulf St Vincent. *Perkinsus* infections were detected in *H. laevigata* collected from reefs south of Edithburgh (Lester 1986). The present investigation was carried out to determine the geographic extent of *Perkinsus* infections in greenlip and blacklip abalone from South Australian coastal waters, and whether *Perkinsus* infections occur in commercially-farmed Pacific oysters *(Crassostrea gigas)* from neighbouring waters.

Materials and Methods

Nine sampling sites were selected from the three abalone fishery management zones of S.A. (Fig. 1, Table 1). Licensed divers collected approx. 30 abalone at random from each site between April and October 1986. A Fisheries Officer also collected approx. 60 oysters from each of four commercial oyster farms. The abalone and oysters were fixed by immersion in Davidson's fluid immediately after collection and the species, sex and shell length were

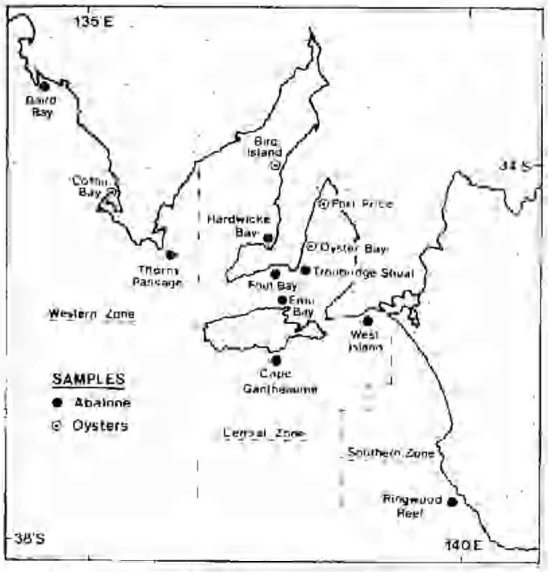


Fig. 1. Locations of sampling sites in South Australian coastal waters from which abalone and oysters were collected.

* Central Veterinary Laboratories, Dept Agriculture, Frome Road, Adelaide, S. Aust. 5000.
† Dept Fisheries, 135 Pirie Street, Adelaide, S. Aust. 5000.

recorded in the laboratory following shell removal. The adductor muscles and mantle were examined for macroscopic lesions on all superficial aspects and internally through a series of longitudinal incisions 1 cm apart. Suspicious lesions were excised together with surrounding tissue. Small blocks of mantle, adductor muscle and gonad tissue were also sampled from each abalone and oyster. Tissues were embedded in paraffin wax, sectioned at 5 μm thickness, stained with haematoxylin and eosin and examined by light microscopy at 100–400 × magnification. Tissue blocks found to contain parasites were then processed for electron microscopy by de-paraffinization in xylol containing 2% osmium tetroxide, clearing in propylene oxide and embedding in epoxy resin (TAAB Laboratories). Ultra-thin sections were cut at 75 nm thickness, stained with 6% uranyl acetate and 0.5% lead citrate and examined in a transmission electron microscope (JEM 100 CX, JEOL, Tokyo). Voucher specimens of fixed tissues containing parasites were deposited with the South Australian Museum, Adelaide (SAM E2180 1).

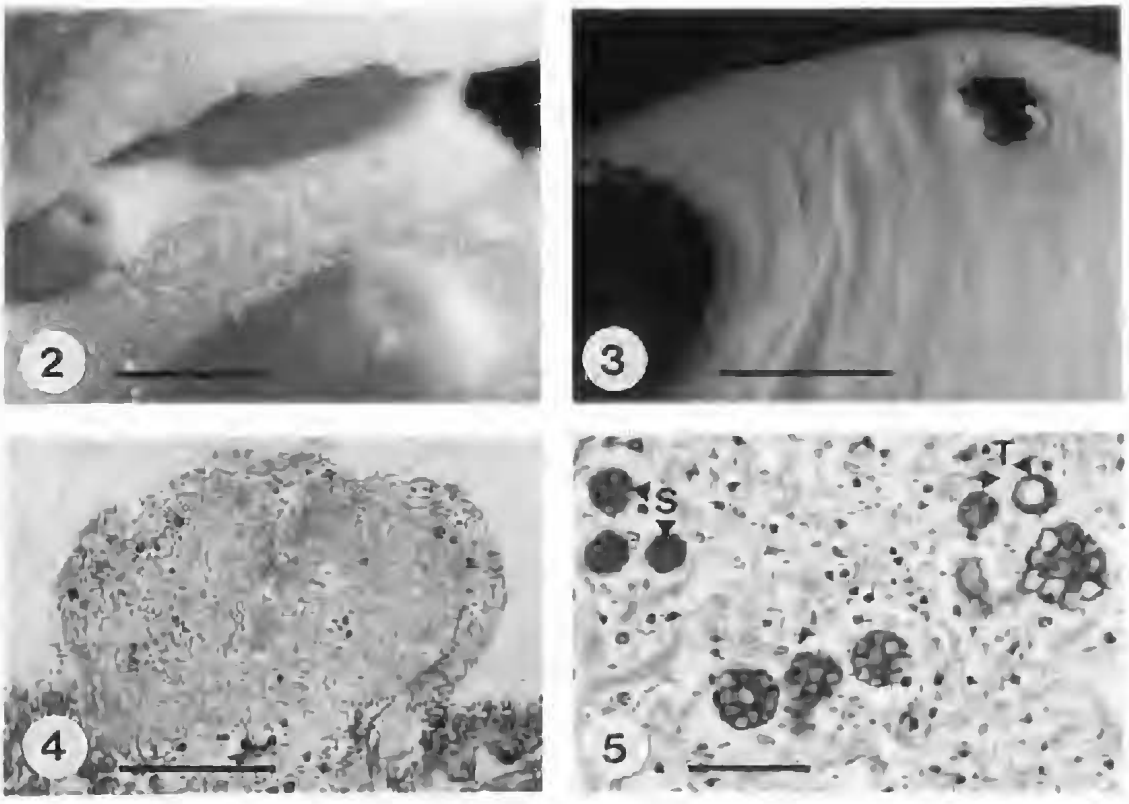
Results

A total of 125 *Haliotis laevigata* and 109 *H. rubra* were collected from the nine sampling sites. *Perkinsus* infections were detected in ten *H. laevigata* (6 ♂♂, 4 ♀♀) collected from Troubridge Shoal in Gulf St Vincent and in nine *H. rubra* (6 ♂♂, 3 ♀♀) from Thorny Passage in Spencer Gulf (Table 1). Infections were not restricted to any particular size (hence age) group of abalone. Infected *H. laevigata* ranged in size from 8.5–16.0 cm in shell length and infected *H. rubra* from 10.0–16.5 cm. No parasitic infections were detected in any of the 240 Pacific oysters examined.

Infections in abalone were characterized by macroscopic hemispherical blister-like nodules on the superficial aspects of the adductor muscles and mantle (Fig. 2). The nodules were soft and slightly darker in appearance than the surrounding tissue. Larger nodules were found to contain creamy viscous fluid when incised. Ovoid nodules were occasionally detected deeper within the tissues when sectioned (Fig. 3). Infection levels ranged from 1–14

TABLE 1. *Prevalence of* Perkinsus *infections in abalone and oysters from South Australia.*

Location	Depth (m)	*Haliotis laevigata* (greenlip abalone)		*H. rubra* (blacklip abalone)		*Crassostrea gigas* (Pacific oyster)	
		No. examined	No. infected	No. examined	No. infected	No. examined	No. infected
Baird Bay (33°08′S, 134°16′E)	10	21	0	23	0	–	–
Thorny Passage (34°58′S, 136°04′E)	5	2	0	16	9	–	–
Hardwicke Bay (34°50′S, 137°22′E)	6	16	0	–	–	–	–
Foul Bay (35°13′S, 137°15′E)	20	8	0	2	0	–	–
Troubridge Shoal (35°08′S, 137°56′E)	5	30	10	–	–	–	–
Emu Bay (35°33′S, 137°34′E)	15	28	0	1	0	–	–
Cape Gantheaume (36°07′S, 137°30′E)	14	–	–	28	0	–	–
West Island (35°37′S, 138°35′E)	10	20	0	15	0	–	–
Ringwood Reef (37°38′S, 140°07′E)	6	–	–	24	0	–	–
Coffin Bay (34°30′S, 135°18′E)	1	–	–	–	–	51	0
Bird Island (33°59′S, 137°33′E)	1	–	–	–	–	52	0
Port Price (34°15′S, 138°04′E)	1	–	–	–	–	66	0
Oyster Bay (34°52′S, 137°48′E)	1	–	–	–	–	71	0
Total		125	10 (8.0%)	109	9 (8.3%)	240	0



Figs 2–5. 2. Nodules on surface of adductor muscle of *Haliotis rubra*. Scale bar = 5 mm. 3. Necrotic lesion in adductor muscle of *H. rubra*. Scale bar = 5 mm. 4. Histological section through nodule in adductor muscle of *H. laevigata*. H&E. Scale bar = 0.5 mm. 5. Trophozoites (T) and schizonts (S) of *Perkinsus* within lesion in adductor muscle of *H. laevigata*. H&E. Scale bar = 50 μm.

nodules per abalone and the nodules ranged in size from 0.5–8.0 mm in diameter. Those detected in *H. laevigata* and *H. rubra* were similar in location, size, shape and appearance. They were not encapsulated but bound by normal tissues which sometimes contained mild infiltrations of mononuclear inflammatory cells (amoebocytes). The nodules were necrotic and contained variable numbers of amoebocytes together with other host cells. The majority of cells appeared degenerative containing pyknotic nuclei. Connective tissue fibres and occasionally the remnants of muscle fibres were found throughout the lesions forming a loose supporting network. All lesions contained numerous clusters of extracellular basophilic bodies which were identified as various developmental stages of a protozoan parasite (Figs 4, 5). The majority of parasite stages were vacuolated in appearance but some were homogeneous and stained uniformly throughout. Most stages appeared degenerative and morphological integrity was not well preserved within lesions. Nonetheless, two types of parasite developmental stages were evident by light and electron microscopy; unicellular and multicellular forms (Fig. 6).

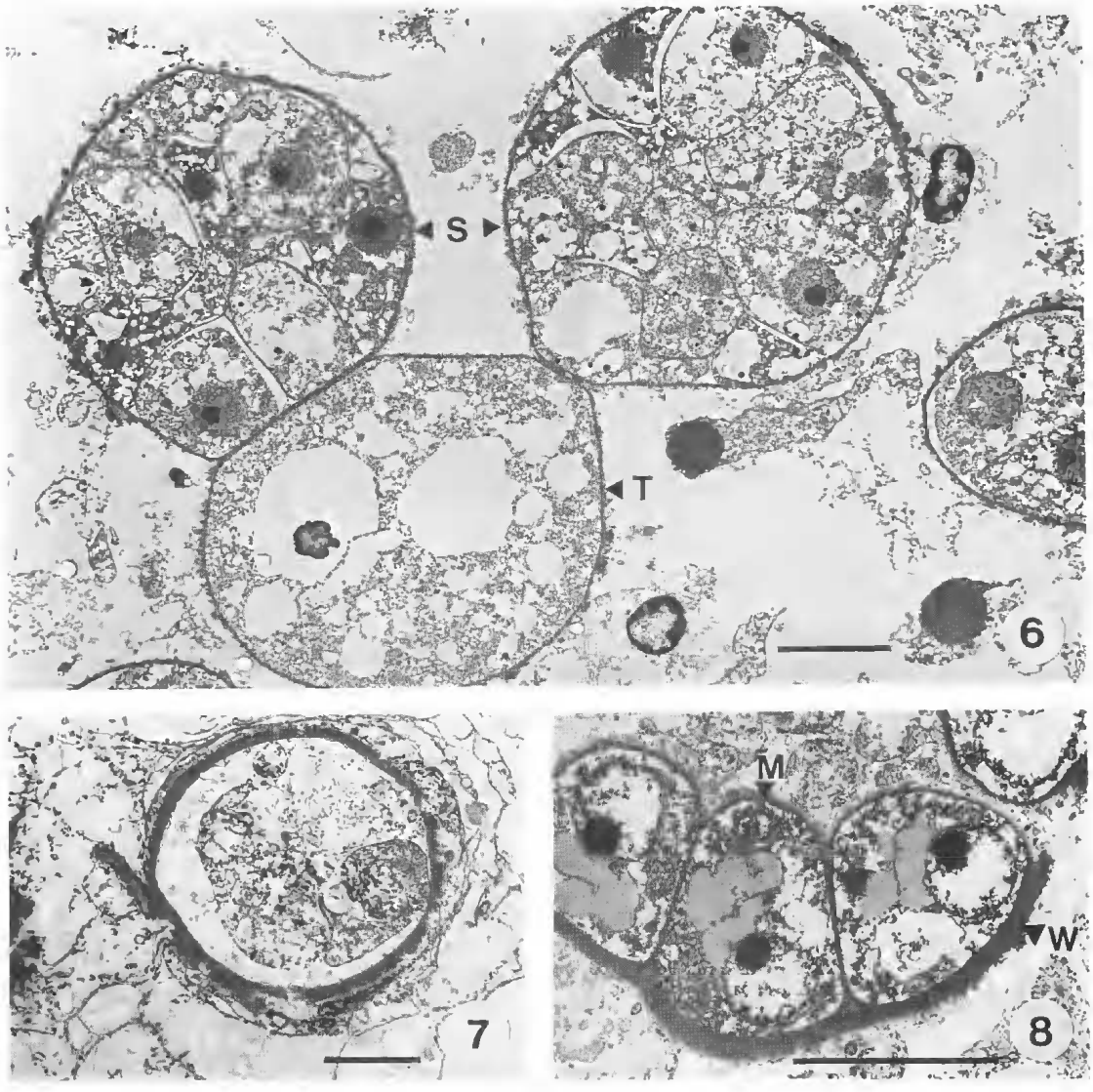
Unicellular stages (termed trophozoites) were found scattered throughout the lesions as individual cells sometimes grouped together in small clusters. They were ovoid in shape measuring from 10.0–17.5 μm in diameter (mean 15.0 μm) and were bounded by a dense wall varying in thickness from 1.5–2.5 μm (Fig. 7). The trophozoites contained a single nucleus with a prominent nucleolus and a highly vacuolated cytoplasm usually containing a large central vacuole measuring from 5–10 μm in diameter. A small dense vacuoplast consisting of eosinophilic granular material was occasionally detected within the central vacuole.

Multicellular stages (termed schizonts) were detected throughout the lesions in distinct clusters (Fig. 8). They were larger in size than the trophozoites measuring from 12.5–35.0 μm in

diameter (mean 27.5 μm). They were surrounded by a dense wall (2.0–3.5 μm thick) with an irregular outer margin. The schizonts contained 2–24 rounded cells (termed merozoites) ranging from 5–10 μm in diameter. Each merozoite contained a highly vacuolated cytoplasm and a single nucleus with a prominent nucleolus. Large central vacuoles were not detected in the merozoites nor were vacuoplasts. The majority of schizonts appeared degenerate particularly towards the centre of the lesion.

Discussion

Identical lesions and parasites detected in *H. laevigata* and *H. rubra* suggests that both abalone species were infected by the same parasite species. This species is similar to *P. olseni* previously reported in blacklip abalone (Lester & Davis 1981). All three parasite developmental stages (trophozoites, schizonts and merozoites) were similar in structure to those previously described although some schizonts appeared larger (mean



Figs 6–8. 6. Electron micrograph of trophozoite (T) and schizonts (S) of *Perkinsus* in adductor muscle of *Haliotis laevigata*. Scale bar = 5 μm. 7. Electron micrograph of *Perkinsus* trophozoite bounded by thick wall. Scale bar = 5μm. 8. Electron micrograph of *Perkinsus* schizont bounded by thick wall (W) and containing several merozoites (M). Scale bar = 5 μm.

diameter of 27.5 μm compared to 15.0 μm) and more mature containing greater numbers of merozoites. However, developing or immature prezoosporangia were not detected and lesions were not surrounded by a loose wall of connective tissue. Despite these differences, the morphological and ultrastructural characteristics of the parasites were considered to be consistent with those of *P. olseni* Lester & Davis, 1981.

Similar developmental stages have been described previously for two other *Perkinsus* spp. Parasites found in the American oyster *C. virginica* were originally described as *Dermocystidium marinum* by Mackin *et al.* (1950) and later as *Labyrinthomyxa marina* by Mackin & Ray (1966). Levine (1978) subsequently renamed the species *Perkinsus marinus* and erected the class Perkinsasida in the phylum Apicomplexa on the basis of the electron microscopic studies of Perkins (1976). This species differs from *P. olseni* in having much smaller trophozoites (3–10 μm in diameter), membranous rather than thickened walls and basophilic rather than eosinophilic vacuoplasts. More recently, thick-walled *Perkinsus*-like trophozoites were found in the gill filaments of the clam *R. decussatus* in Portugal by Comps & Chagot (1987) and Chagot *et al.* (1987). These parasites were cultured in thioglycolate medium to form mature sporangia containing biflagellated zoospores by Azevedo (1989) and were named *P. atlanticus* on the basis of host identity, pathology and zoospore ultrastructure. The dimensions, shape and flagellar organization of the zoospores were more regular than those of *P. marinus* but comparisons with *P. olseni* could not be made because their zoospore ultrastructure has not yet been determined. Several other undescribed *Perkinsus* spp. have been reported in 57 species of molluscs from North America, the Mediterranean and Australia (Andrews 1954; Ray 1954[1]; da Roz & Canzonier 1985; Goggin & Lester 1987) but comparisons could not be made because the only developmental stages reported were large ovoid cells presumed to be prezoosporangia.

Early cross transmission studies suggested that *Perkinsus* spp. may be specific for particular groups of molluscs; *P. marinus* for oysters (lamellibranchs) and *P. olseni* for abalone (gastropods) (Ray 1954[1]; Lester & Davis 1981). However, recent studies have not supported any rigid host specificity for these parasites. *P. olseni* isolated from *H. laevigata* was successfully transmitted to two lamellibranch species *(Pinctada sugillata* and *Anadara trapezia)* and *Perkinsus* spp. isolated from five lamellibranchs *(Anadara trapezia, Chama pacificus, Tridacna gigas, T. crocea* and *T. maxima)* were successfully transmitted to *H. laevigata* (Goggin *et al.* 1989). *P. marinus* has also been transmitted from the oyster *C. virginica* to the pyramidellid gastropod *Boonea impressa* (White *et al.* 1987). These results suggest that *Perkinsus* infections may be transmitted between different mollusc species inhabiting the same waters. No infections were detected in oysters sampled from neighbouring areas in this study but other mollusc species remain to be examined.

Infected *H. rubra* and *H. laevigata* were detected at two different sites located 140 km apart in adjacent Spencer Gulf and Gulf St Vincent. Infections have previously been found in abalone from the same general areas (Lester & Davis 1981; Lester 1986). The reasons for this patchy distribution of infections are not known. The two sites are separated by Yorke Peninsula but both are situated near the mouths of the Gulfs where the same ocean current passes in an easterly direction. However, no infections were detected in abalone sampled from intermediate sites nor from sites located further away in the same current flow. There are also no records of abalone stocks being moved between the two sites of infection. These sites must be regarded as potential point sources of infection and local mollusc populations should be monitored regularly for the spread of infections.

Significant mortalities of *H. laevigata* were first reported along the western coast of Gulf St Vincent in 1980 and further deaths were reported each summer from 1982–1985 (Lewis *et al.* 1987). Abalone had been abundant in this area as far north as Black Point but stocks have now practically disappeared (K.L. Branden pers. comm.). Claims made by divers that mortalities were due to pollution were not substantiated by laboratory investigations for heavy metals, organochlorines, organophosphates and hydrocarbons (Shepherd 1985). Subsequent studies revealed that many abalone in this area were infected with *P. olseni* (Lester 1986) but it is not known whether infections caused the mortalities. The parasite is certainly pathogenic and causes necrotic lesions within host tissues. Mortalities have been observed in experimentally infected *H. rubra* maintained in the laboratory at 20°C whereas those maintained at 15°C recovered from infection (Lester 1986). The continued detection of *Perkinsus* infections in abalone from dieback areas highlights the need for further studies on parasite pathogenicity, transmission and control.

[1]Ray, S. M. (1954) Biological studies of *Dermocystidium marinum*, a fungus parasite of oysters. Rice Institute Pamphlet, Special Issue. Unpubl.

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